


Human Journals

Research Article

May 2016 Vol.:3, Issue:3

© All rights are reserved by K.Tejaswini et al.

Formulation and Evaluation of Fluconazole Loaded Transfersome Gel



IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals



K.Tejaswini*, Swapna.S¹, A.Madhu Babu², Vasudha Bakshi³

**,1,2,3 Department of Pharmaceutics, Anurag Group of Institutions (Formerly Lalitha College of Pharmacy), Venkatapur (v), RangaReddy-500088, Telangana, India.*

Submission: 29 April 2016
Accepted: 7 May 2016
Published: 25 May 2016



HUMAN JOURNALS

www.ijsrm.humanjournals.com

Keywords: Transfersome, Fluconazole, Spans, Thin film hydration, Rotary evaporation

ABSTRACT

Objective: Transdermal drug delivery has made an important contribution to medical practice, but has yet to fully achieve its potential as an alternative to oral delivery and hypodermic injections. Various new technologies have been developed for the transdermal delivery of some important drugs. The goal of the present study was to formulate and evaluate the potential use of transfersomal vesicles as a transdermal drug delivery system for the poorly soluble drug, Fluconazole. **Methods:** It was investigated by encapsulating the drug in various transfersomal formulations composed of various ratios of different spans (80,60,40) and tween-80 prepared by thin film hydration method. The prepared formulations were characterized for entrapment efficiency (EE%), drug content, in-vitro skin permeation studies and stability studies. **Results:** The vesicles were spherical in structure as confirmed by Transmission Electron Microscopy. The EE% of Fluconazole in the vesicles was in the range of 90.84%. The result revealed that Fluconazole in all of the formulations was successfully entrapped with uniform drug content. A transfersomal gel containing 20mg of the drug and 20% of span 80 was concluded as the optimized formulation (F5), as it showed maximum drug entrapment (90.84%) and cumulative percent drug release (88.52%). Further stability studies were carried out at $25 \pm 2^\circ\text{C}$ for a period of 4 weeks. **Conclusion:** It is evident from this study that transfersomes are a promising prolonged delivery system for Fluconazole and have reasonably good stability characteristics. This research suggests that Fluconazole loaded transfersomes can be potentially used as a transdermal drug delivery system.

INTRODUCTION

Fluconazole is a synthetic antifungal agent belonging to the group of triazole used in the treatment of oropharyngeal, esophageal, or vaginal and urinary tract infection as well as other serious systemic candidal infections. It is also effective against superficial fungal infections and dermatophytoses. Fluconazole is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, bloating and abdominal discomfort. Accounting these problems, drug delivery technologies should be developed which reduces drug dosing frequency along with sustained or controlled release of the medicament as well as reduced systemic side-effects ^[1].

The vesicular drug-carrier system, transfersome have been reported to enhance the transdermal delivery of drugs, when applied onto the skin non-occlusively. Transfersomes have the ability to overcome the permeation difficulty by squeezing themselves along the inter-cellular sealing lipid of the stratum corneum. The resulting flexibility of transfersomes from membranes minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, after application onto the skin. ^[2]

Transdermal delivery of anti-fungal proved to be a convenient route of administration for a variety of clinical indications. In addition, using the gel as a delivery system can increase the residence time of drugs on the skin and provide a faster release of drug substance ^[3].

Fluconazole is a polar bis-triazole antifungal drug. The presence of two triazole rings (bis-triazole) makes this compound less lipophilic and more hydrophilic when compared with other azoles antifungal agents. The presence of halogenated phenyl ring increases its antifungal activity.

It is a BCS class III drug (high solubility and low permeability). On oral administration, its bioavailability is low due to poor aqueous solubility. Fluconazole was selected for the formulation of topical transfersomal gel because patients with diseases candidiasis and urinary tract infection etc., the dose is given at a higher level due to its low permeability. The ethosomal approach was selected to enhance the permeability of fluconazole that increases bioavailability, reduce the side effects, reduce large doses and increase the therapeutic efficacy.

MATERIALS AND METHODS

Fluconazole was received as a gift sample from Virupaksha laboratories Ltd., Hyderabad, India. Soya lecithin was obtained from Bright laboratories. Tween 80, Span 80, 60, 40 were obtained from Merck specialties pvt. Limited (Mumbai). All other chemicals used in this study were of analytical grade.

Preparation of Fluconazole loaded transfersomes ^[4,5]

Twelve Transfersome formulations were prepared by thin film hydration method using Fluconazole, Soya Lecithin, and different surfactants (Tween-80, Span-80,60,40). The amount of drug is kept constant (100mg) for all the formulations (Table 1). Lecithin, surfactants, and the drug are dissolved in 5ml of organic solvent (ethanol) and then placed in a clean, dry bottom flask. The organic solvent was carefully evaporated by rotary evaporation under reduced pressure above the lipid transition temperature to form a lipid film on the wall of the flask and lipid film was hydrated with a phosphate buffer solution (pH 7.4) by rotation for 1hr at room temperature at 60 rpm. The resulting vesicles are swollen for 2 hrs at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated using probe sonicator (Heldolph vcx750) for 30 minutes at 40°C.

Table 1: Formulation chart of formulations F1-F12

Formulation code	Fluconazole (mg)	Soya Lecithin (mg)	Tween 80 (mg)	Span 80(mg)	Span 60(mg)	Span 40(mg)
F1	100	90	10	-	-	-
F2	100	85	15	-	-	-
F3	100	80	20	-	-	-
F4	100	90	-	10	-	-
F5	100	85	-	15	-	-
F6	100	80	-	20	-	-
F7	100	90	-	-	10	-
F8	100	85	-	-	15	-
F9	100	80	-	-	20	-
F10	100	90	-	-	-	10
F11	100	85	-	-	-	15
F12	100	80	-	-	-	20

Compatibility study of drug and polymer using FTIR ^[6]

The compatibility between the pure drug and excipients was detected by FTIR spectra obtained on Bruker FTIR Germany (Alpha-T-1020). The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in². The spectra's were recorded over the wave number of 3500 to 500cm⁻¹.

Characterization of transfersomes:

Vesicle shape ^[7]

Transfersomes vesicles can be visualized by optical microscope. The morphological characterization of transfersome vesicle such as shape and surface features were projected by using an optical microscope. A drop of transfersome suspension was placed over the slide and Photomicrograph was taken at 10x resolution.

Vesicle size, size distribution, and zeta potential analysis ^[8,9]

The average diameter and size distribution profile and zeta potential analysis of Vesicles were determined by Zetasizer. Particle size and zeta potential of transfersomes in the dispersion were determined by photon correlation spectroscopy (PCS) using Malvern zeta sizer at a fixed angle of 90° at 25 °C using water as a dispersant for size determination and zeta potential measurement.

Determination of pH of Transfersome gel ^[10]

The value of pH of topical transfersome gels was measured by using a digital pH meter (Labindia Sab 5000 pH meter) at the room temperature.

Determination of percentage entrapment efficiency ^[11,12]

The amount of Lornoxicam entrapped in transfersome gel was estimated by centrifugation method. 1gm of Transfersome gel was taken and diluted with 10ml phosphate buffer (pH 7.4). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was

placed in a centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of the supernatant was withdrawn and diluted 20 times before going for absorbance measurement using UV spectrophotometer (UV-3200 Lab India) at 261nm. This gives us the total amount of unstrained drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$E.E\% = [(C_t - C_f) / C_t] \times 100$$

% Drug content ^[13]

1gm of a transfersome gel formulation was taken and the vesicles were lysed with 25 ml of ethanol by sonication for 15 min. Later this solution was placed in a centrifugation tube and centrifuged at 14000 rpm for 30 minutes. The clear solution was diluted to 100 ml with methanol. Then 10 ml of the solution was diluted to 100 ml with phosphate buffer pH7.4. Aliquots were withdrawn and drug content was calculated for Lornoxicam by using UV spectrophotometer at 261nm.

***In vitro* drug release studies through cellophane membrane** ^[14]

The *in vitro* permeation behaviour of Lornoxicam from all transfersomal gel formulations and were investigated using cellophane membrane (Molecular weight cut off 12000–14000, HI Media Ltd, Mumbai, India). Vertical type of the Franz diffusion cell was used for the permeation study. The cellophane membrane was mounted on a diffusion cell assembly with an effective diffusion area of 2 cm². The receptor compartment consisted of a 30 ml phosphate buffers at pH 7.4 as the receptor fluid agitated at 100 rpm and was maintained at 37 ± 0.5°C throughout the experiments. The prepared formulation was applied to the membrane in the donor compartment. An aliquot of 2 ml sample was withdrawn at suitable time intervals and replaced immediately with an equal volume of fresh diffusion medium. The cumulative amount that permeated across the cellophane membrane was calculated and plotted against time

Drug release kinetics ^[15]

To understand the drug release kinetics of the Lornoxicam gel formulation, the drug release data were treated as zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Korsmeyer-Peppas equation. If the value of „n“ is less than 0. 5

then it is considered as a Fickian release, values more than 0.45 and less than 0.89 is considered as anomalous (non- Fickian) transport and finally „n“ value greater than 0.9 follow super case-II release mechanism.

Stability Studies^[16]

The stability studies were carried out according to % entrapment efficiency and drug content at $25\pm 2^{\circ}\text{C}$ for a period of 30 days.

RESULTS AND DISCUSSION

Results:

Formulation of transfersomes:

Span 80 was selected as the edge activator surfactant for the transfersomal formulation as it is biocompatible and pharmaceutically acceptable. Phospholipid was used as the bilayer-forming agent and ethanol was used as the hydrating agent because ethanol is known to extract stratum corneum lipids and alter the barrier property of the intracellular lipoidal route, thereby allowing higher drug permeation.

Drug Excipient Compatibility Study

Drug Excipient Compatibility studies were performed using FTIR spectrophotometer. The IR spectrum of pure Lornoxicam and physical mixture of drug and other excipients i.e., the optimized formulation was studied. The correlation between the pure drug and excipients indicated that the drug was compatible with the formulation excipients.

FTIR data of the pure drug and the drug with excipients (span 80 and soya lecithin) suggested that there was no interaction between the drug and the excipients used.

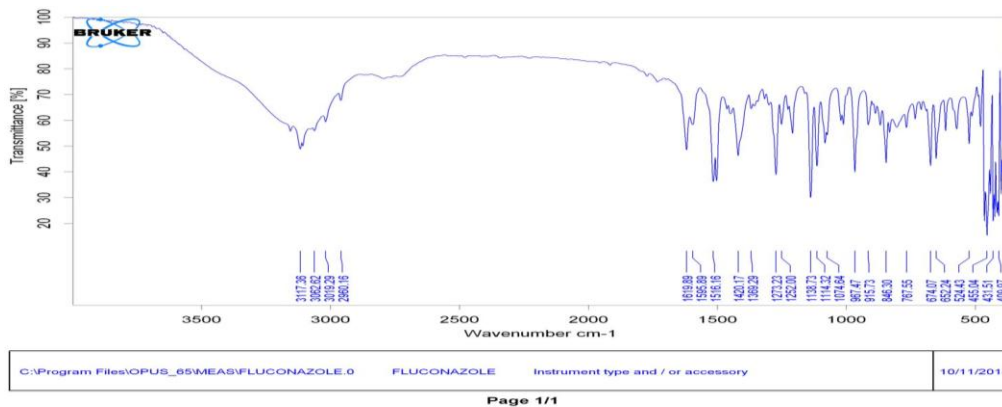


Figure 1: FTIR Spectra of Fluconazole pure drug

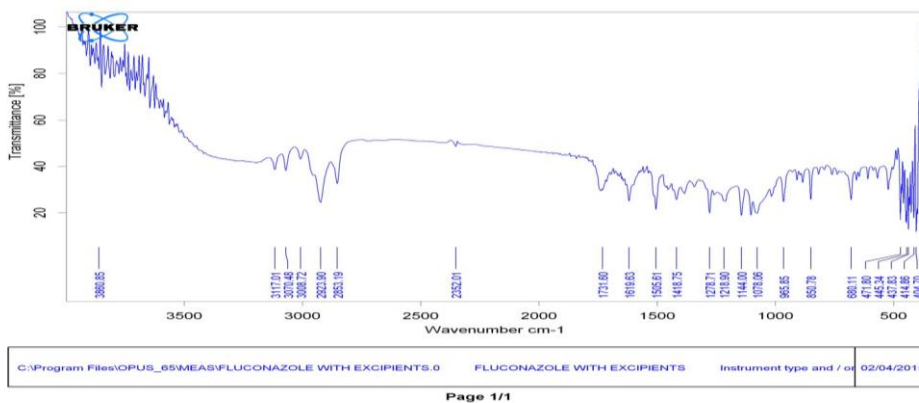


Figure 2: FTIR Spectra of Fluconazole with excipients

Vesicle shape and type

The surface morphology was studied by Optical microscopy and transmission electron microscopy. The shapes of most of the Fluconazole containing transfersomes were found to be spherical, as shown in figure 3

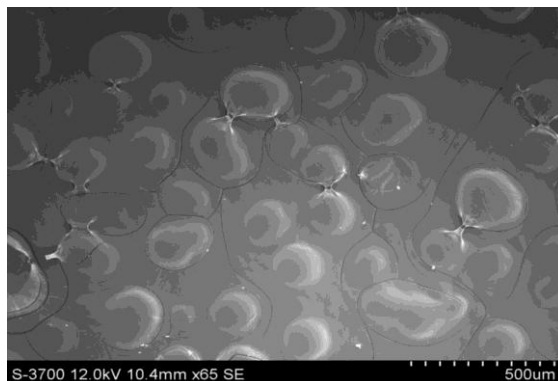


Figure 3: Photomicrograph of Fluconazole loaded transfersome (F5) at 10X

Vesicle size, size distribution zeta potential

The vesicle size, size distribution, and zeta potential of the optimized formulation were determined by light scattering method using Zetasizer (DTS Version 5.03, Malvern). The mean vesicle diameter was found to be 163.5nm and zeta potential was found to be -13.8mV. Size distribution curve confirms the normal size distribution of the vesicles.

Table 2: Z-Average size, zeta potential and PDI of Optimized formulation

Particle size & Z-Potential analysis			
Formulation	Z-Average size (d.nm)	PDI	Z-Potential (mV)
F5	163.5	0.251	-13.8

pH value of topical transfersome gel

The value of pH of topical transfersome gels was measured by using a digital pH meter (LabindiaSab 5000 pH meter) at the room temperature. The pH of all topical transfersomal gels was found to be in the range of 7.4 ± 0.02 to 7.4 ± 0.08 .

Entrapment efficiency

% entrapment efficiency of deformable vesicles formulations was found to be in the range of 82.71 ± 0.074 to 90.84 ± 0.078 .

Percentage entrapment of Fluconazole was found to be maximum with the formulation of F5(maximum 90.84 ± 0.074 for F5) because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The entrapment efficiency of drug decreased when the molar ratio of lipid to surfactant was decreased from 90:10 to 80:20. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, Fluconazole, the efficiency decreased with increasing surfactant concentration and thus increased with increasing lipid concentration.

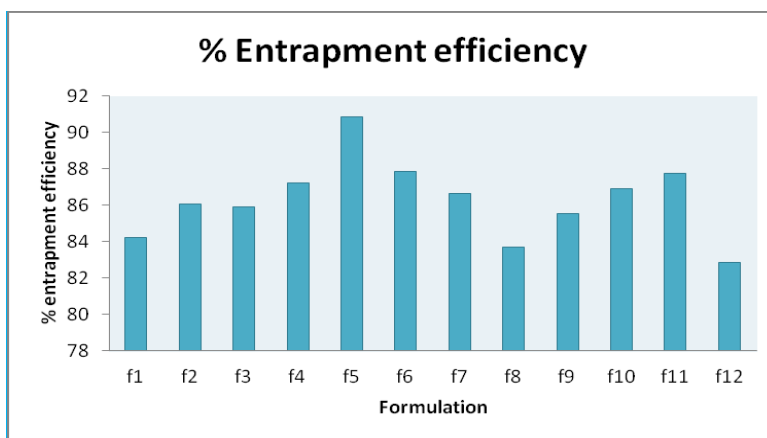


Figure 4: Entrapment efficiency of all formulations

Percentage (%) Drug content:

The results obtained shows 82.9-90.6% drug content in all the formulations (Figure 5), which shows that there is no degradation of the drug in the process.

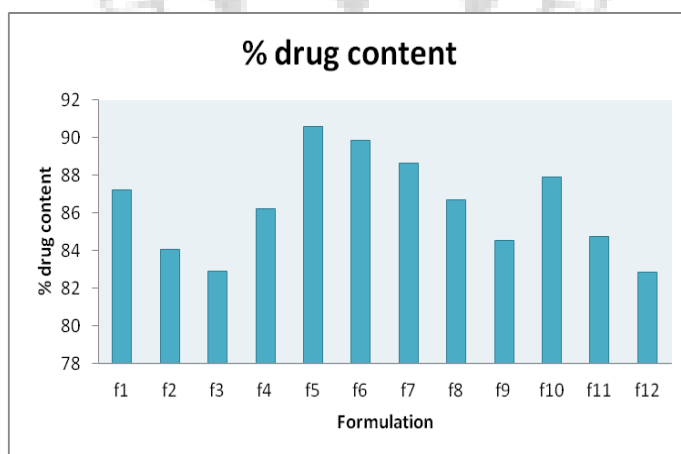


Figure 5: % Drug content of formulations f1-f12

In vitro drug release studies through a cellophane membrane

Each transdermal gel formulation was subjected to *in vitro* drug release studies using a cellophane membrane. The cumulative amount of drug release was calculated for each formulation. Results revealed that the f5 (formulation with 20% span 80) had the highest cumulative amount of drug release (87.5%) up to 12 hrs as compared to other transdermal gel formulations. The release rate of fluconazole from f5 was significantly higher than the other

formulation (Figure 6 & 7). The release experiments clearly indicated controlled-release of fluconazole from the transfersomal gel formulation. The maximum release was observed in f5, because of the higher drug content and entrapment efficiency of the formulation. The maximum release was also due to optimum surfactant concentration (20%), because at this concentration the surfactant molecule gets associated with the phospholipid bilayer resulting in better partitioning of the drug, and resulted in higher drug release from the vesicles.

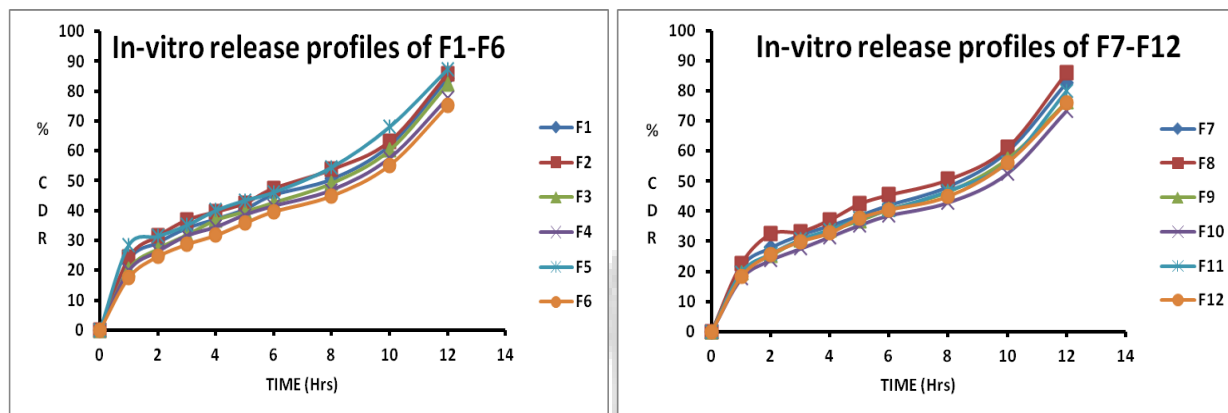


Figure 6: *In-vitro* release profiles of F1-F6 Figure 7: *In-vitro* release profiles of F7-F12

Drug release kinetics

The drug release data were explored for the type of release mechanism followed. Release kinetic study of all formulation (f1 to f12) was studied for different kinetic equations (zero order, first order, Higuchi, and Korsmeyer-peppas equation).

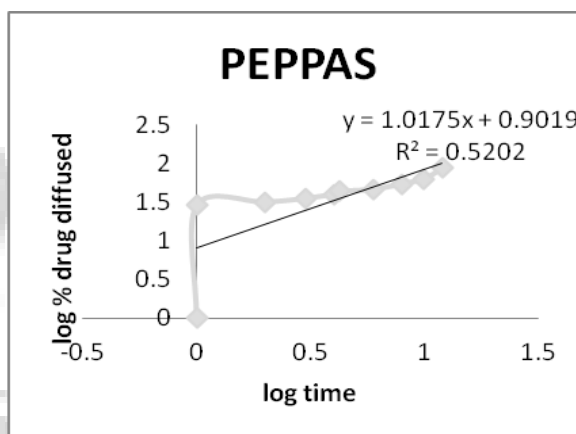
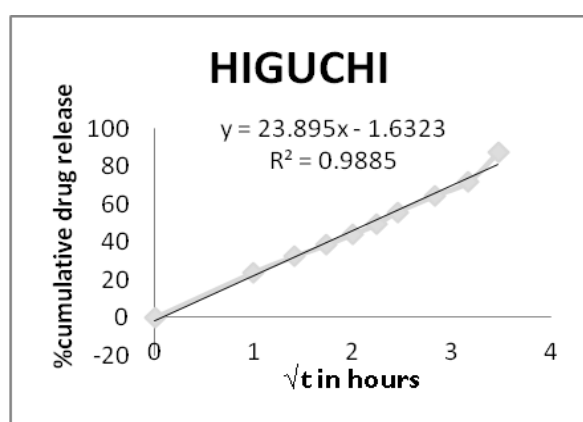
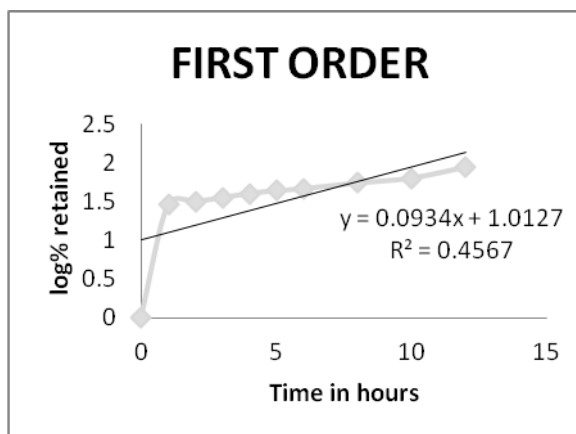
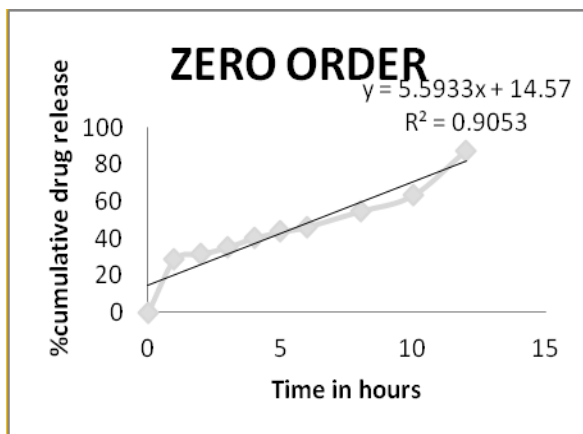
Perusal in Table 3 indicates that R^2 values for the formulations F1 to F12 were found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism. According to Korsmeyer-peppas model, slope (n) value less than 0.45 indicates the Fickian diffusion. From the results obtained all the formulations (F1-F12) having the slope (n) value between 0.351 and 0.442. This indicates that the formulation follows the release mechanism of Fickian diffusion. Considering the correlation coefficient (R^2) values obtained from the different kinetic equations, the drug released from most of all formulations (F1 to F12) were found to follow zero order and Higuchi release model (Table 3).

Table 3: Drug release kinetics data of all formulations

Formulations	Zero order (r²)	First order (r²)	Higuchi (r²)	Korsemeyer - Peppas (r²)	n value
F1	0.891	0.716	0.852	0.763	0.375
F2	0.842	0.760	0.873	0.817	0.419
F3	0.862	0.820	0.894	0.846	0.351
F4	0.861	0.708	0.867	0.758	0.429
F5	0.905	0.456	0.988	0.520	0.426
F6	0.859	0.859	0.862	0.873	0.414
F7	0.846	0.809	0.884	0.823	0.410
F8	0.860	0.769	0.889	0.813	0.395
F9	0.869	0.842	0.921	0.862	0.442
F10	0.849	0.832	0.905	0.853	0.441
F11	0.880	0.774	0.861	0.821	0.432
F12	0.878	0.858	0.892	0.872	0.413

Table 4: release kinetics data for formulation F5

Parameter	Zero order	First order	Higuchi	Korsemeyer- Peppas
Regression values [R²]	0.905	0.456	0.988	0.520
Slope	5.593	0.093	23.89	1.017
Intercept	14.57	1.012	1.632	0.901



Stability studies

It is clear from the results obtained that the transfersomes have shown the minimum drug loss at room temperature, and fairly high retention of the drug inside the vesicles was observed. At this temperature condition % remaining drug entrapped and drug content was good over a period of 30 days.

Table 5: % Entrapment efficiency and % Drug content after stability studies

Number of days	% Entrapment Efficiency		% Drug content	
	Before (25±2 ⁰ C)	After (25±2 ⁰ C)	Before (25±2 ⁰ C)	After (25±2 ⁰ C)
30 days	90.84±0.985	88.74±1.854	90.6±0.58	89.32±0.987

Based on the above data, it was confirmed that prepared fluconazole transfersome gel (f5) can be considered as a good approach to reduce the dosing frequency and to maintain the drug concentration at the desired site of application.

CONCLUSION

Fluconazole is an triazol derivative used as an anti-fungal agent. It is well absorbed following oral administration however, its use has been associated with a number of undesirable side effects. Fluconazole is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, bloating and abdominal discomfort. Oral fluconazole cannot be taken in conjunction with a number of medications. In order to bypass these disadvantages, the Transfersosomal gel formulations have been proposed as a topical application. Despite not worthy advances have been made over recent years in the management of fungal diseases, the currently available methods, have a dose limiting therapeutic index with compromised safety implications. To alleviate this problem, vesicular drug delivery system transfersomes is formulated to deliver Fluconazole across skin and target drug to synovium or specific tissues which in turn increase drug efficacy with minimum extra synovial toxicity. Finally, it can be concluded from the results of the present study that transfersome gel improves the transdermal delivery, prolong the release, and improve the site specificity of the drug Fluconazole. Transfersomes formed from Lecithin: Span 80 is a promising approach to improve the permeability of Fluconazole in a period of time. Transfersomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

ACKNOWLEDGMENT

The authors are thankful to the Chairman Dr. P. Rajeshwar Reddy and Principal Dr. Vasudha Bakshi of Anurag Group of Institutions, Formerly Lalitha College of Pharmacy, Ghatkesar, Telangana for permitting to carry out research work

REFERENCES

1. Debjit B and Harish G, "Recent advance in topical drug delivery system." The Pharma Innovation .2012,1,13-31.
2. Kim A, Lee EH, Choi SH, Kim CK. In vitro and in vivo transfection efficiency of a novel ultra deformable cationic polymer. *Biomaterials*.2004; 25: 305–313. [http://dx.doi.org/10.1016/S0142-9612\(03\)00534-9](http://dx.doi.org/10.1016/S0142-9612(03)00534-9) .

3. Patel MR, Patel RB, Parikh JR, Solanki AB, Patel BG. Effect of formulation components on the in vitro permeation of microemulsion drug delivery system of fluconazole. *AAPS PharmSciTech*. 2009; 10: 917–923.
4. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization, and evaluation of transdermal insulin delivery. *Saudi Pharm J*. 2012; 20: 355–363.
5. Davuluri C and Rayudu N, “Vesicular systems emerging as Dermal and Transdermal Drug Delivery Systems.” *International Journal of Pharmaceutical Science Review and Research*, 2013, 18(1), 176-182.
6. Patel N and Patel S, “Review on: A Vesicular transdermal delivery system for enhancing drug Permeation Ethosomes and Transferosomes.” *International Pharmaceutical Sciencia*. 2012, 2, 24-34.
7. Swathi K and Bavanapriyadarshini K, “Transferosomes: A Review.” *An International Journal of Advances in Pharmaceutical Sciences*. 2013, 4, 125-133.
8. Prajapati S.T, Patel C.G and Patel C.N, “ Transferosome: A vesicular carrier system for Transdermal drug delivery.” *Asian journal of Biochemical and Pharmaceutical Research*, 2011, 1, 507-524.
9. Pandey S, Goyani M, Devmurari V and Fakir J, “Transferosomes: A Novel Approach for Transdermal Drug Delivery”, *Scholars Research Library Der Pharmacia Lettre*. 2009, 1(2), 143-150.
10. Avinash K, Dwivedi A and Sunda M, “Transferosome: The drug loaded ultradeformable vesicles for transdermal drug delivery”, *International Research Journal of Pharmacy*. 2011, 2(11), 40-20.
11. Modi C.D and Bharadia P.D, “Transferosomes: New Dominants for Transdermal Drug Delivery”, *American Journal of Pharmtech Research*. 2012, 2(3), 71-91
12. Nathji D and Patni C, “Transferosome: An enhancement approach for transdermal drug delivery system.” *International journal of pharmacy and integrated life sciences*. 2012, 12, 14-29.
13. Patel P and Patel U, “Review on transferosome.” *World Journal of Pharmacological Research and Technology*. 2013, 1(1), 25-31. Impact factor: 0.3397/ICV: 4.10 311.
14. Bhoomi et al. / *Pharma Science Monitor* 5(2), Sup-1, Apr-Jun 2014, 298-312.
15. Goodman and Gilman’s, *The pharmacological basis of therapeutics*, 10th edn McGraw Hill Med. Pub. Div. New York, 2001, pp 804.
16. Bhalaria M.K, Naik S and Mishra A.N, “Ethosomes: A novel delivery system for antifungal drugs in the treatment of topical fungal diseases.” *Indian Journal of Experimental Biology*. 2009, 47, 368-375.
17. Doaa A., Dalia R, Sally H and Mohamed N, “ Formulation and evaluation of fluconazole topical gel.” *International journal of pharmacy and pharmaceutical sciences*. 2012, 4, 176-183.

HUMAN